

Further Evaluation of the Automated Fluorescent Treponemal Antibody Test for Syphilis

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Improvements in the equipment for the automated fluorescent treponemal antibody (AFTA) test for syphilis prompted this comparative study of the AFTA and its manual counterpart, the fluorescent treponemal antibody-absorption (FTA-ABS) test. The AFTA equipment operated satisfactorily, required only minimal monitoring, and afforded a three-to fourfold increase over the number of sera that could be tested manually by one serologist. The AFTA and FTA-ABS tests agreed well with only 2.1% of the sera yielding conflicting results. The AFTA was less precise than the FTA-ABS on sera retested because of original conflicting results and on sera retested within the same run to determine reproducibility. However, these differences were not large, and AFTA test performance was considered to be within the limits acceptable for a diagnostic serological procedure.

The SeroMatic System developed by Aerojet-General Corp. (1) provides a tool for doing large numbers of indirect fluorescent antibody tests with a considerable saving in staff time over that required for manual methods. This system adapted for the automated fluorescent treponemal antibody (AFTA) test was evaluated at the Venereal Disease Research Laboratory (3), in this laboratory (2), and in the health department laboratories of two other states. Mechanical problems encountered in these studies led to modifications of the equipment. The purpose of the present study was to evaluate the modified equipment by comparing its performance in the AFTA test with that of the manual fluorescent treponemal antibody-absorption (FTA-ABS) test on sera submitted to this laboratory for FTA-ABS testing.

MATERIALS AND METHODS

Test procedure. The provisional technique for the AFTA test was used (5). The FTA-ABS procedure developed at the Venereal Disease Research Laboratory (4) was employed, except that slides were prepared on the day of use and fixed in 10% methanol for 5 min (6).

Reagents. Reagents for the AFTA test were generously supplied by the Aerojet-General Corp. Antigen for the FTA-ABS test was prepared in this laboratory (6); other reagents were obtained from commercial sources.

Equipment. The AFTA test employed the SeroMatic System (R) comprising an electropneumatically controlled slide processor and a microscope stage attachment (1).

Slides processed for either procedure were examined with a Zeiss fluorescence assembly with an Osram HBO200 mercury lamp in combination with a Zeiss microscope equipped with a dark-field condenser, a BG-12 exciter filter, and a no. 50 barrier filter.

Test sera. Five hundred and twenty-one individual human sera were tested independently by both the AFTA and FTA-ABS methods. The only criteria for selection of specimens were that they had been submitted for FTA-ABS testing and possessed sufficient volume to be tested by both procedures. Sera were stored at 6°C until testing was completed.

Reproducibility sera. As a measure of within run reproducibility, 49 sera were tested in duplicate by the AFTA procedure and 41 sera in duplicate by the FTA-ABS test.

Control sera. Control sera for the AFTA test were obtained from the manufacturer. FTA-ABS control sera were prepared in this laboratory.

RESULTS

The reactions obtained in the AFTA and FTA-ABS tests are given in Table 1; the AFTA test yielded five more reactive and eight more borderline results than were found in the FTA-ABS test. A comparison of the reactivity of the two tests was carried out. The AFTA and FTA-ABS tests agreed, giving the same reactions in 456 (87.5%) of the 521 sera tested (227, reactive; 4, borderline; 225 nonreactive). Partial agreement was obtained in an additional 54 sera (10.4%) in which 14 of these sera were reactive (AFTA, 11; FTA-ABS, 3) in one test and borderline (AFTA, 3; FTA-ABS, 11) in the other, and 40 were nonreactive (AFTA, 12 nonreactive; FTA-ABS, 28 nonreactive).

tive) in one test and borderline in the other (AFTA, 28; FTA-ABS, 12). Complete disagreement between the results of the AFTA and FTA-ABS tests was found in 11 sera (2.1%); 4 were reactive only in the AFTA test and 7 only in the FTA-ABS test. Only 6 of the 11 specimens showing complete disagreement contained sufficient serum volume to repeat both the AFTA and FTA-ABS tests; these results are shown in Table 2. When repeat testing verified the original result of one test, but not the other, the unverified original test result was considered to have been in error. There were three such errors in the AFTA test and one in the FTA-ABS test. Disagreements between the two tests persisted upon retesting the remaining two specimens.

As a measure of test reproducibility, approximately every 11th specimen was retested by inserting it at random within the next set of 10 specimens. The differences between the duplicate tests are shown in Table 3. In the AFTA test, 59% of the sera tested in duplicate gave the same plus reading, 96% agreed within ± 1 plus, and no differences were greater than 2 plus. In the FTA-ABS, 80% of the duplicate tests gave the same reading and all agreed within ± 1 plus. It is possible that reader bias may have influenced the reproducibility of the FTA-ABS test more than

the AFTA. In the AFTA test the reader was not aware that a serum was being retested to determine reproducibility until after the slide was read. In the FTA-ABS test, although the reader scrupulously avoided examining the previous result, he was aware at the time the slide was read that the serum was being retested to determine reproducibility.

DISCUSSION

The AFTA test, by automating many of the time-consuming aspects of the manual FTA-ABS test, provides a tool for substantially increasing the number of sera that can be tested by one serologist. As part of a national field trial, the AFTA test had previously been evaluated in this laboratory by comparing its performance with that of the FTA-ABS test on sera from clinically defined donor groups (2). Although the results of the two tests disagreed in 3.5% of the sera tested, the majority of these differences were not true, repeatable differences between the two tests but were apparently due to test error, with 2.7% of the sera yielding such errors in the AFTA test and 0.6% in the FTA-ABS test. These results were achieved with continuous monitoring of the AFTA slide processor and with manual correction of observed mechanical errors. Since that study was completed, changes have been made in the slide processing equipment to improve test performance and eliminate the need for constant monitoring. Lewis and his associates at the Venereal Disease Research Laboratory reported that changes in the equipment had substantially improved the accuracy of the AFTA test (3).

The study reported here was undertaken to evaluate the improved equipment on sera sub-

TABLE 1. Reactivity of AFTA and FTA-ABS tests

Test	Total sera	Reactive		Borderline		Nonreactive	
		No.	Per cent	No.	Per cent	No.	Per cent
AFTA	521	242	46.5	35	6.7	244	46.8
FTA-ABS	521	237	45.5	27	5.2	257	49.3

TABLE 2. Original and repeat test results on sera giving conflicting results in the AFTA and FTA-ABS tests

Test	AFTA run no.	Serum no.	Original test results ^a		Repeat test results	
			AFTA	FTA-ABS	AFTA	FTA-ABS
Original AFTA result not verified by repeat test	II	0313	R (2+)	N	N	N
	IV	1395	N	R (4+)	R (4+)	R (4+)
	IV	1845	N	R (4+)	R (3+)	R (3+)
Original FTA-ABS result not verified by repeat test	I	4752	R (3+)	N	R (2+)	R (2+)
Both results verified by repeat test	VI	3122	N	R (1+)	N	R (2+)
	VII	3231	R (3+)	N	R (2+)	N

^a Abbreviations: R, reactive; N, nonreactive.

TABLE 3. Within-run reproducibility of AFTA and FTA-ABS tests

Range of reactivity of sera (pluses)	AFTA duplicate tests				FTA-ABS duplicate tests			
	No. of sera	Reading difference			No. of sera	Reading difference		
		None	±1	±2		None	±1	±2
3-4	10	3	6	1	7	6	1	
2-3	11	2	8	1	10	4	6	
1-2	4	2	2		2	1	1	
0-1	1	1			1	1		
0	23	21	2		21	21		
Total	49	29	18	2	41	33	8	0
Per cent		59	37	4		80	20	0

mitted to this laboratory for FTA-ABS testing. The automated equipment operated well, required only minimal monitoring, and yielded more satisfactory results than in the previous study. Although exact agreement between the AFTA and FTA-ABS tests was obtained in only 87.5% of the 521 sera tested, an additional 10.4% gave borderline reactions (6% in the AFTA and 4.4% in the FTA-ABS). The two tests disagreed, one testing being reactive and the other nonreactive, in only 2.1% of the sera. Repeat testing by both the manual and automated procedures of 6 of these 11 sera verified the original FTA-ABS result in 5 sera and the original AFTA result in 3 sera. The automated test was less precise than the manual procedure in duplicate tests done to measure reproducibility. However, these differences were not large, and, in our opinion, the performance of the AFTA test in this study was within the limits acceptable for a diagnostic serological procedure.

The serologists doing the test preferred the automated to the manual procedure because of the ease of processing slides and especially because of the facilitated reading. We estimate that a three- to fourfold increase over the number of sera tested manually by one serologist can be achieved with the automated equipment. We would recommend that individuals planning to substitute the AFTA for the FTA-ABS test first undertake parallel testing with both procedures on sera representative of the type to be tested. This will establish the relative precision of the two procedures for that laboratory and provide information necessary for intralaboratory control and for interpretation of AFTA test results. The results of the comparative study reported here substantiate the conclusion of Lewis and his associates (3) that the AFTA test can now provide an effective tool for serological testing.

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